§ 113.312 Rabies Vaccine, Live Virus.

Rabies Vaccine shall be prepared from virus-bearing cell cultures or embryonated chicken eggs. Only Master Seed Virus which has been established as pure, safe and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in § 113.300.

(1) Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(2) Each lot of Master Seed Virus propagated in tissues or cells of avian origin shall be tested for pathogens by procedures prescribed in § 113.37.

(3) Each lot of Master Seed Virus propagated in primary cell cultures of mouse or hamster origin or brain tissues of mouse origin shall be tested for lymphocytic choriomeningitis (LCM) virus by the procedure prescribed in § 113.42. If LCM virus is detected, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be studied in each species of carnivore or domesticated wild animal for which the vaccine is specifically recommended to attempt to determine the fate of the vaccine virus. Results shall be considered in evaluating safety of vaccine virus.

(i) Obtain at least 10 unvaccinated animals, negative at 1:2 final serum dilution, of each species in which tests will be conducted. Divide each species into two groups of five animals.

(ii) For each species of animal, inject one group of five animals intramuscularly. Infiltrate a major nerve and the surrounding tissue in each of the five animals in the other group. Use 1.0 ml of high titer virus for each method of administration.

(iii) Observe all animals for signs of rabies until scheduled time to sacrifice. If animals show definite symptoms, sacrifice and check regional lymph nodes, brain, salivary glands, and kidney for rabies virus by injection of suckling mice (not more than 7 days of age). Tissues may be held frozen at −70 °C. until suckling mice are available. Inject each mouse in one litter intracerebrally with 0.02 ml of a ground tissue suspension from each organ. Observe mice each day for 21 days. If any mice die, determine if the deaths were due to rabies virus in the brain by a fluorescent antibody test.

(iv) Sacrifice animals that do not show signs of rabies according to the following schedule and check regional lymph nodes, brain, salivary glands, and kidney in suckling mice.

Route of injection	Days after injection	Number of animals
Intramuscularly	15, 20, 25, 30, 35	1 each day.
Intraneurally	3, 6, 9, 15, 30	1 each day.

(5) Each lot of Master Seed Virus shall be tested for safety in at least 10 unvaccinated serologically negative animals of each domestic species for which the vaccine is recommended.

(i) Each group of 10 animals shall be divided into 2 groups of 5 animals. For each species, inject one group intramuscularly with 10 doses of high titer virus.

(ii) Infiltrate a major nerve of each of the animals in the other group of 5 with 10 doses of the same high titer virus. For all species except dogs and cats, multiple injections along the cervical spine in the proximity to the nerve trunks emerging from the spinal cord may be used: *Provided*, That a 1-dose volume shall be injected into each of four or more sites bilaterally.

(iii) Observe all animals each day for 90 days.

(iv) If any animals show clinical signs of rabies, sacrifice the animal and check appropriate brain tissue for rabies virus by the fluorescent antibody test and by mouse injection.

(v) If rabies is confirmed, the lot of Master Seed Virus is unsatisfactory.

(b) The immunogenicity of vaccine prepared with virus at the highest passage of the Master Seed shall be established in each species for which the vaccine is recommended. Tests shall be conducted in accordance with a protocol filed with Animal and Plant Health Inspection Service before initiation of the tests. The vaccine shall be prepared using methods prescribed in the Outline of Production. If Rabies Vaccine is to be in combination with other fractions, the product tested

shall include all fractions to be recommended.

(1) A geometric mean virus titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(2) The dose of vaccine to be used in the immunogenicity test shall be no more than the amount of rehydrated vaccine which, on the basis of previous titrations, has been diluted to the proposed minimum acceptable virus titer.

(3) Test animals shall be uniform and have no neutralizing antibodies to rabies as determined by serum-neutralization (SN) tests.

(i) Twenty-five or more animals shall be used as vaccinates. Each shall be injected intramuscularly at one site in the thigh with a dose of vaccine at the proposed minimum virus titer as specified in the filed Outline of Production.

(ii) Ten or more additional animals shall be held as controls.

(iii) On or about days 30, 90, 180, 270, and 365 postvaccination, all animals shall be bled and individual serums tested for neutralizing antibodies to rabies virus.

(iv) All surviving test animals of each species shall be challenged intramuscularly with virulent rabies virus furnished or approved by Animal and Plant Health Inspection Service 1 year after vaccination, except as provided in paragraphs (b)(4), (b)(5), and (b)(6) of this section. The challenged animals shall be observed each day for 90 days as prescribed in §113.5(b). The brain of each test animal that dies following challenge shall be examined for rabies by the fluorescent antibody test or other method acceptable to Animal and Plant Health Inspection Service.

(v) Requirements for acceptance in challenge tests shall be death due to rabies in at least 80 percent of controls while at least 22 of 25 or 26 of 30 or a statistically equivalent number of the vaccinates remain well for a period of 90 days.

(4) An alternative to challenging all surviving test animals in accordance with paragraph (b)(3)(iv) of this section may be used when the test animals are of species other than carnivores. Vaccinates shall be challenged at 1 year postvaccination. These shall include five vaccinates with the lowest SN titers at the 270th-day bleeding, five vaccinates with the lowest SN titers at the 365th-day bleeding, and all vaccinates with SN titers below 1:10 by the mouse SN test or below 1:16 by the rapid-fluorescent-focus-inhibition test at any bleeding. At least five SN-negative controls of each species shall be challenged at the same time as the vaccinates. All SN titers shall be iterated to an endpoint. All of the challenged vaccinates must remain well for a period of 90 days, and at least 80 percent of the controls must die of rabies for a satisfactory test without further challenge. If one or more of the vaccinates die from rabies, all the remaining vaccinates, regardless of titer, along with the five controls shall be challenged. The cumulative results from the two challenges shall be evaluated for acceptance as specified in paragraph (b)(3)(v) of this section.

(5) An outline of Production change shall be made before authority for use of a new lot of Master Virus shall be granted by Animal and Plant Health Inspection Service.

(c) If more than 1 year duration of immunity is to be claimed, a duration of immunity test for the additional time shall be conducted and interpreted as prescribed in paragraph (b) of this section for the 1 year test. The test animals shall be monitored serologically at least every 180 days. The time of challenge may be adjusted accordingly.

(d) Test requirements for release: Each serial and each subserial shall meet the general requirements prescribed in §113.300 and special requirements in this paragraph.

(1) *Purity and safety tests.* Final container samples of completed product from each serial or one subserial shall be tested.

(i) The test for pathogens, prescribed in §113.37 shall be conducted on each serial or one subserial of avian origin. If necessary, neutralize the rabies virus with specific rabies antiserum.

(ii) A test for safety in three young seronegative animals of the most susceptible species for which the vaccine is recommended shall be conducted. Each shall be injected intramuscularly with 10 recommended doses of vaccine. If unfavorable reactions attributable to the product occur during a 28 day observation period, the serial is unsatisfactory.

(iii) If primary cell cultures of hamster origin or of mouse origin are used vaccine production, they shall be tested for LCM virus as prescribed in §113.42. The cells shall be disrupted and undiluted cell fluids from each lot shall be tested.

(2) *Virus titrations.* Final container samples of completed product shall be tested for virus titer using the titration method used in paragraph (b)(1) of this section. To be eligible for release, each serial and each subserial shall have a virus titer sufficiently higher than the titer of the vaccine virus used in paragraph (b) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer equal to or greater than that used in the immunogenicity test.

(3) Young adult mice, each weighing 14 to 16 grams, shall be used as test animals when the virus in vaccine prepared with a low egg passage Flury Strain or high cell passage Street Alabama Dufferin Strain (HCP SAD) of rabies virus is titrated. At least 10 mice for each dilution shall be used.

(i) At least 10 mice shall be used for each dilution. Each shall be injected intracerebrally with 0.03 ml.

(ii) The injected young adult mice shall be observed each day for 14 days except when testing vaccines made with HCP SAD strain of rabies virus, in which case, the mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD_{50} titer calculated by the Reed and Muench Method.

(iii) Virus titer requirements for release and at expiration date shall be determined for each vaccine on the basis of data available: *Provided*, That, the lowest titer permitted at expiration date when determined by this test shall be $10^{3.0}$ LD_{50} per 0.03 ml.

(4) Suckling mice, 6 days of age or younger, shall be used as test animals when virus in vaccine prepared with a high egg passage Flury Strain of rabies virus is titrated.

(i) Six to twelve mice shall be used for each dilution. Each shall be injected intracerebrally with 0.02 ml.

(ii) The injected suckling mice shall be observed each day for 21 days. Deaths and paralysis occurring subsequent to the fourth day post-injection shall be noted and the LD_{50} titer calculated by the Reed and Muench Method; and

(iii) Virus titer requirements for release and at expiration date shall be determined for each vaccine on the basis of data available: *Provided*, That, the lowest titer permitted at expiration date when determined by this test shall be $10^{3.0}$ LD_{50} per 0.02 ml.

[39 FR 44721, Dec. 27, 1974, as amended at 40 FR 20067, May 8, 1975; 42 FR 6795, Feb. 4, 1977; 43 FR 49529, Oct. 24, 1978; 50 FR 20090, May 14, 1985; 50 FR 23797, June 6, 1985. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991; 61 FR 31823, June 21, 1996; 72 FR 72564, Dec. 21, 2007]

§113.313 Measles Vaccine.

Measles Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable general requirements prescribed in §113.300. Each lot of Master Seed Virus shall meet the special requirements prescribed in this section.

(b) To detect virulent canine distemper virus, each of two canine distemper susceptible ferrets shall be injected with a sample of the Master Seed Virus equivalent to the amount of virus to be used in one dog dose and observed each day for 21 days. If undesirable reactions occur in either ferret, the lot of Master Seed Virus is unsatisfactory.

(c) Each lot of Master Seed Virus used for vaccine production shall be